Effects of Modified Diet on Suppression of Disease Activity of Rheumatoid Arthritis

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Abstract

To investigate the efficacy of a vegetarian diet (VD) enriched with n-3 polyunsaturated fatty acids (PUFAS) & n-9 monounsaturated fatty acids (MUFAS) versus an omnivorous normal diet (ND) for suppression of disease activity in patients with acute and active rheumatoid arthritis (RA).

Eighty (80) patients with active and acute RA for at least six months duration with disease activity 28 score (DAS 28 score) of 3.2 or more were enrolled for the study. Each of the patients received MTX & non-steroidal anti-inflammatory drugs (NSAIDs). All of them were randomly allocated either to VD or ND. To compare the nutritional influence of this modified vegetarian diet, 20 healthy persons were also enrolled to the VD as control group.

Clinical examinations, laboratory immunological, biochemical, hematological and radiographic analysis were performed at baseline, and again in the 1st, 3rd, & 6th months. A composite disease activity index (DAS 28), Ritchie's articular index score for RA, Physical function index (Functional index of Lee), quality of life reported by short form 36 (SF-36) health survey, daily consumption of NSAIDs, visual analogue scale(0-10cm) of pain (pain VAS),ESR(mm/1st hour-Westergren) were used as clinical efficacy variables. Plasma levels of C-reactive protein (CRP), IgG & IgM rheumatoid factors, Interleukine-1 beta (IL-1β) and Tumor Necrosis Factor alpha (TNF- α) were used as immunological efficacy variables. Total plasma protein, Fibrinogen, cholesterol & triglyceride, Beta- Lipids, body mass index (BMI) and hemoglobin concentration (Hb) were used as nutritional parameters.

From baseline to the end of the study , the patients in VD group showed a significant difference of change from respective baseline values in DAS 28 score(P<0.05),Ritchie's articular index score(P<0.05), functional index of Lee score (P<0.05), SF-36 health survey parameters (P<0.05), daily NSAIDs consumption (P<0.05), pain VAS (P<0.05), ESR (P<0.05), CRP (P<0.05), IgG RF (P<0.05), IgM RF (P<0.05), IL-1 beta (P<0.05), Hb (P<0.05), plasma protein (P<0.05), Fibrinogen (P<0.05), cholesterol (P<0.05) & triglyceride

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($p<0.05$), LDL ($p<0.05$), HDL ($p<0.05$) compared to ND group; but not for TNF- α ($P>0.05$) & BMI ($P>0.05$). And in comparison to healthy control group though Hb ($p<0.05$), plasma protein ($P<0.05$), Fibrinogen ($P<0.05$), Cholesterol ($P<0.05$) & LDL ($P<0.05$) was changed significantly in VD group but the change of triglyceride ($P>0.05$), HDL ($P>0.05$) & BMI ($P>0.05$) was not significant. Improvement of disease activity in between VD & ND group indicated significant difference from the 3rd months & onwards.

The results indicate that patients with active and acute RA, by adjusting to a vegetarian diet fortified with n-3 PUFAS & n-9 MUFAS, did obtain a reduction in inflammatory activity with improvement in level of well being with minimal influence on nutritional status.

Key words: Rheumatoid arthritis, Modified Diet, n-3 polyunsaturated fatty acid, n-9 monounsaturated fatty acid.

Introduction

The treatment of RA is traditionally characterized by repeated changes in medication. The first step is non-steroidal anti-inflammatory drugs (NSAIDs), and if necessary a sequence of progressively toxic second line drugs (disease modifying anti-rheumatic drugs) was administreted.[1] There is evidence that some of these diseases modifying drugs provide a degree of decrease[2] in disease activity but also maintain or improve physical function and retard radiographic joint damage[3]. However, both patients and physicians are not satisfied with the long-term results of traditional therapy. In a study in 1996 suggested that early introduction of disease- modifying anti rheumatic drugs may be more beneficial than delayed introduction for patients with recently diagnosed RA.[4] Research was focused towards finding new, more effective drugs, re-assessment and earlier use of existing drugs such as (corticostesiods[5]), treatment with drug combinations[6], and dietary modifications.[7,8]

Literature supports that dietary factors such as olive oil[2] and cooked vegetables[9] have positive influence on relieving the symptoms of patients suffering from RA.[9] Controlled trial of fasting followed by a vegetarian diet in RA indicates that dietary treatment can reduce disease activity in some patients with rheumatoid arthritis.[10,11,12] Fasting followed by lacto-vegetarian diet also have positive influence on the management of R.A.[13] Case control studies indicate that lifelong consumption of olive oil[2,9] cooked vegetables[9] and fasting followed by vegetarian or lacto-vegetarian diet[10,11,12,13] may have independent protective effects on the development or severity of RA. Epidemiological studies from selected geographical regions support these hypotheses. In north western Greece where the consumption of olive oil is high, the prevalence of RA has been reported to be low.[14] It is intriguing for rheumatologists to note that alpha-linolenic acid rich diet, in secondary prevention of coronary heart disease, was reported to reduce the recurrence rate of new cardiac events.[15] The pathogenesis of atheromatosis involves inflammatory processes[16] with obvious similarities to those of rheumatoid synovitis.[7] In the atherosclerotic plaque microenvironment, as in RA synovitis, macrophages are the principal inflammatory mediators with the ability to form numerous growth factors and cytokines.[7] Nutritional support, in the form of lipids and antioxidants, may act at the foci of modulation of

cytokine biology by nutrients and amino acid provision may alter the availability of substrate for the production of proteins and peptides which are important in the inflammatory process.[17] Beneficial dietary manipulation of cytokine production and actions may be designed to facilitate, enhance, or suppress events, depending on the biological or clinical context in which they are operating.[18] Studies using animal models have illustrated that manipulation of the intake of a wide range of nutrients can modulate many deleterious effects of infective and inflammatory states.[19] In this proposed trial of a vegetarian diet supplemented with soybean oil and olive oil which are rich in anti-oxidants , n-3 polyunsaturated fatty acid(PUFAS) & n-9 monounsaturated fatty acid (MUFAS), and low in 'rich sources' of proteins and peptides was supposed to or expected to reduce the clinical and immunological duration of activity of rheumatoid arthritis.

Patients and Methods

Patients: A total of 100 subjects were enrolled for this study. Eighty patients were recruited from out patient department for rheumatology patients of Department of Internal Medicine including Rheumatology and Metabolism & Department of Physical Medicine, Ibrahim Memorial BIRDEM Hospital, Dhaka, in collaboration with St-Petersburg medical academy of post-graduate studies, St-Petersburg, Russia, and, Institute of Rheumatism, Nutrition & Environmental Disorders, Dhaka, Bangladesh from January 2004 to January 20005. Twenty healthy persons specially the relatives / attendant of the patients of same age group without having arthritis, diabetes mellitus, metabolic syndrome-x were recruited from various areas of Dhaka city specially at Nandipara, Bashabo, Dhaka, Bangladesh- as control group by home visits after explaining them the study protocol for motivating them to abide by the terms and conditions of the study.

The inclusion criteria for the patients were: R.A. according the American College of Rheumatology - 1987 revision criteria for the diagnosis of R. A. [20], Age between 35 to 65 years, A disease duration of more than 6 months, A disease activity score from 28 joints (DAS 28 score) had to be 3.2 or more indicating active disease [21,22] .

A number of exclusion criteria prevented patients from participating - patients suffering from other medical disease including metabolic, endocrine, hepatic, renal, hematological, pulmonary or cardiovascular disease, disease duration of less than 6 months, Patients who were already vegetarian, DAS-28 score less than 3.2. [22].

All patients and healthy control subjects were informed orally and in writing about the study design, the underlying hypothesis, and right for the participant to withdraw from the project at any time, and for whatever reasons. And written consent was obtained.

Study Design: The study design was a multi-center, randomized, parallel study. Primarily all of 100 selected subjects were divided into two groups- experimental patient group and healthy control group. The experimental patients were again divided into two groups (fourty patients in each group), with age ranged between 35-65 years and having same disease activity & duration of RA. The patients of the first

group (VD group) were given a vegetarian diet enriched with soybean oil and olive oil, non-steroidal anti-inflammatory drugs (NSAIDs-namely Diclofenac sodium/ Indomethacin/Naproxen) and methotrexate (MTX) (7.5mg/ week). The second group (ND group) was allowed to take usual omnivorous normal diet, NSAIDs & MTX as mentioned earlier. Clinical examinations, laboratory immunological, biochemical, hematological and radiological conditions of the patients of both groups & healthy controls were analyzed once at the entry, after one month, after 3 months and again after 6 months of study simultaneously with this complex therapy. Healthy control group were given only modified vegetarian diet. Evaluations of type and dosages of NSAIDS comprised a 7 days recall NSAIDs questionnaire.

Table 1: Baseline characteristics of patients and healthy control group who were in the trial. Data are presented as mean. (SD)

	Modified diet +MTX (VD group)		Normal diet +MTX (ND group)		Control	
Characteristics	Male N=12	Female N=28	Male N=13	Female N=27	Male N=3	Female N=17
Age in years	53.42 (8.61)	44.07 (5.71)	47.00 (8.70)	46.30 (6.07)	50.00 (1.73)	39.88 (4.62)
Height in meter	1.60 (0.11)	1.54 (0.05)	1.59 (0.09)	1.54 (0.05)	1.65 (0.14)	1.53 (0.05)
Weight in kg	64.88 (8.14)	57.89 (5.59)	60.09 (4.24)	61.11 (4.43)	65.00 (5.80)	60.55 (4.41)
BMI	25.33 (2.05)	24.57 (2.60)	23.97 (2.77)	25.90 (2.49)	23.95 (2.63)	25.74 (1.57)
Disease duration in month	19.42 (4.44)	20.32 (4.45)	20.08 (4.37)	19.14 (5.10)	-	-
RA test (sero-positivity/ negativity)	28 (70%)/12 (30%)		31 (77.5%) /9 (22.5%)		-	
Amount of NSAIDs equivalent Dicofenac (mg/day)	114.23 (3.52)		115.19 (3.77)		-	
Fasting blood sugar (m.mol/l)	4.16 (0.49)		4.33 (0.41)		4.50 (0.25)	

Table 2: Clinical indices of diseases activity at baseline and months 1, 3 and 6; Data are presented at mean (SD)

	Modified diet + MTX (VD group)				Normal diet MTX (ND group)				P values in between VD&ND		
	Base Line N=40	Month 1 N=40	Month 3 N=35	Month6 N=33	Base Line N=40	Month 1 N=40	Month 3 N=37	Month 6 N=35	Month 1	Month 3	Month 6
DAS28 score (2-10)	7.23 (0.44)	6.69 (0.48) P<0.05	5.50 (0.39) P<0.05	4.24 (0.39) P<0.05	7.29 (0.39)	6.93 (0.39) P<0.05	5.93 (0.40) P<0.05	4.85 (0.54) P<0.05	P<0.05	P<0.05	P<0.05
Ritchie's Articular index (0-68)	32.09 (3.99)	25.10 (4.28) P<0.05	17.42 (4.17) P<0.05	8.10 (2.93) P<0.05	33.25 (4.28)	28.13 (4.18) P<0.05	21.03 (4.23) P<0.05	12.74 (4.71) P<0.05	P<0.05	P<0.05	P<0.05
Functional indices of Lee (0-40)	25.98 (3.27)	21.63 (3.70) P<0.05	15.09 (3.61) P<0.05	7.06 (2.68) P<0.05	26.93 (3.47)	24.48 (3.75) P<0.05	18.16 (3.59) P<0.05	11.06 (4.08) P<0.05	P<0.05	P<0.05	P<0.05
Pain VAS 0-10mm	6.58 (0.91)	5.16 (0.92) P<0.05	3.58 (0.90) P<0.05	1.66 (0.59) P<0.05	6.75 (1.01)	5.71 (0.97) P<0.05	4.29 (0.94) P<0.05	2.59 (1.01) P<0.05	P<0.05	P<0.05	P<0.05
Thrombocyte Count ($x10^5$/l)	292.83 (14.48)	258.35 (16.03) P<0.05	262.37 (11.81) P<0.05	260.12 (5.70) P<0.05	296.25 (15.53)	268.65 (13.07) P<0.05	272.81 (12.31) P<0.05	267.14 (5.74) P<0.05	P<0.05	P<0.05	P<0.05
WBC count ($X 10^3$/l)	10.52 (0.77)	10.00 (0.70) P<0.05	7.19 (1.13) P<0.05	7.04 (0.59) P<0.05	10.75 (0.87)	10.46 (0.59) P<0.05	8.15 (1.14) P<0.05	7.81 (0.65) P<0.05	P<0.05	P<0.05	P<0.05
ESR(westergren) (mm/1st h)	75.78 (14.69)	65.18 (12.99) P<0.05	54.80 (13.48) P<0.05	29.70 (7.32) P<0.05	76.40 (14.75)	68.50 (15.34) P<0.05	60.62 (16.05) P<0.05	42.26 (13.43) P<0.05	P>0.05	P>0.05	P<0.05
NSAID's / Equivalent diclofenac (mg/day)	114.23 (3.52)	95.80 (9.16) P<0.05	51.67 (9.76) P<0.05	43.36 (10.83) P<0.05	115.19 (3.77)	101.83 (7.84) P<0.05	60.40 (10.22) P<0.05	57.49 (12.03) P<0.05	P<0.05	P<0.05	P<0.05

P values refer to level of significance to difference between modified diet and normal diet groups for the changes from baseline to months 1,3 & 6.Difference between groups were analyzed by student's t test for independent samples except for Ritchie's articular index score, Functional index of lee score, pain VAS evaluated by Mann-whitney U test. Within group differences at months 1,3 & 6 compared with baseline, were evaluated by student's t test for paired sample except for Ritchie's articular index score, Functional index of lee score, Pain VAS which were evaluated by Wilcoxon signed ranked test.

Dietary Intervention: The usual omnivores diet group (ND) patients received no dietary advice apart from encouraging them to take sufficient amount of normal omnivorous diet. Patients in vegetarian diet group (VD) and normal healthy control advised by the research rheumatologist and nutritionist to adopt a modified vegetarian type of diet.

Compliance with vegetarian (VD) and omnivorous (ND) diet in respective group of patients were ascertained by questionnaire, and by dietary history interviews. The questionnaire was designed to examine food choice and it was specifically be aimed at

investigating compliance with the modified vegetarian diet. Soybean oil was allowed for food preparation, baking & cooking and olive oil for salad dressing. No recommendations were given about alcohol consumption. To compensate for the polyphenol present in wine, patient was encouraged to take green or black tea (if needed).Each patient was visited fortnightly. At each subsequent visit, a dietary survey and further counseling was done. Diet evaluations comprised a 24 hours recall and a frequency questionnaire.

Table 3: Quality of life reported by experimental patients and healthy control groups. Data are presented as mean (SD).

	Modified diet + MTX (VD group)			Normal diet +MTX (ND group)			Healthy control group			P values in between VD & ND groups	
	Base line N=40	Month 3 N=35	Month 6 N=33	Base line N=40	Month 3 N=37	Month 6 N=35	Base line N=20	Month 3 N=20	Month 6 N=20	Month 3	Month 6
Physical function	48.12 (7.23)	60.92 (7.06) P<0.05	68.25 (7.35) P<0.05	46.77 (6.32)	54.03 (7.55) P<0.05	60.82 (6.74) P<0.05	87.99 (0.30)	87.83 (0.53) P>0.05	87.89 (0.37) P>0.05	P<0.05	P<0.05
Physical role	28.28 (3.85)	57.01 (9.74) P<0.05	74.10 (7.11) P<0.05	27.95 (3.77)	53.51 (11.44) P<0.05	63.64 (9.58) P<0.05	83.26 (0.52)	83.46 (0.54) P>0.05	83.12 (0.37) P>0.05	P>0.05	P<0.05
Bodily pain	33.48 (3.56)	58.34 (6.26) P<0.05	65.85 (4.56) P<0.05	32.94 (3.85)	54.20 (6.06) P<0.05	61.38 (4.26) P<0.05	74.42 (0.35)	74.22 (0.34) P>0.05	74.40 (0.37) P>0.05	P<0.05	P<0.05
General health	56.34 (0.72)	63.98 (2.66) P<0.05	70.03 (1.98) P<0.05	56.26 (0.69)	61.54 (2.73) P<0.05	67.92 (2.41) P<0.05	75.79 (0.37)	75.57 (0.28) P>0.05	75.50 (0.64) P>0.05	P<0.05	P<0.05
Vitality	47.60 (0.64)	55.99 (1.50) P<0.05	64.17 (4.18) P<0.05	47.51 (0.55)	54.28 (1.53) P<0.05	59.34 (5.64) P<0.05	68.83 (0.49)	68.55 (0.49) P>0.05	68.65 (0.39) P>0.05	P<0.05	P<0.05
Social functioning	64.83 (1.86)	69.85 (5.59) P<0.05	72.11 (6.75) P<0.05	64.54 (1.51)	65.04 (4.06) P>0.05	65.90 (4.07) P<0.05	88.57 (0.32)	88.44 (0.42) P>0.05	88.66 (0.35) P>0.05	P<0.05	P<0.05
Emotional role	51.04 (1.11)	52.69 (1.37) P<0.05	52.72 (0.86) P<0.05	50.93 (1.02)	51.30 (1.32) P<0.05	51.44 (1.22) P<0.05	85.56 (0.60)	85.40 (0.74) P>0.05	85.73 (0.65) P>0.05	P<0.05	P<0.05
Mental health	66.27 (1.26)	74.61 (1.10) P<0.05	79.22 (0.78) P<0.05	66.27 (1.23)	73.41 (1.04) P<0.05	78.28 (0.88) P<0.05	80.62 (0.56)	80.58 (0.68) P>0.05	80.40 (0.53) P>0.05	P<0.05	P<0.05

P values refer to level of significance to difference between modified diet group & normal diet group for the changes from baseline to months 3 & 6. Differences within group were evaluated by wilcoxon signed ranks test and in between groups by student's t test.

Clinical Assessments: DAS 28 score was used for clinical assessment of disease activity. [21,22] Ritchie's Articular index joint score was used for assessment about the tenderness of joints which is a single, reliable and clinically convenient measure of pain in inflamed joints in patients with rheumatoid arthritis[23]. The joint-tenderness index representing the sum for joints graded according to following grades- grade 0 – the patient had no tenderness; grade +1 – the patient complained of pain; grade +2 – patient complained of pain and winced; grade +3 – the patient complained of pain, winced & withdrew. Total possible scores are fifty (50) [23]. The version-2 of the short form – 36 health survey (SF-36) was used for the patients to report health related quality of life. The test measures multidimensional health concepts including levels of well being.[24] Patients overall functional status was assessed by using standard questionnaire of present functional index of lee. The maximum total score possible was 40.[25] Total numbers of swollen joints were counted among 28 joints. The total number of tender joints were also counted among 28 joints with tenderness on pressure or pain on passive motion (or both).[21]General Health status of patient was measured by using patients global assessment of visual analogue scale (patient global VAS, 0 – 100mm)on a horizontal visual analogue scale, worst & best imaginable health status at the right and left anchor respectively.[21]Patients evaluated their own pain severity on a visual analogue scale VAS (pain VAS, 1 – 10mm) with worst & imaginable pain at the right achor.[26,17]Duration of morning stiffness was assessed in minutes.[26]

Immunological, Biochemical & Hematological Analysis: Analyses were carried out from the samples of blood which were obtained from the patient in all groups at study entry (base line), after one month, three months and six months of study. Biochemical, a few immunological namely RA test & C-reactive protein and hematological analysis were carried out immediately after the blood samples were drawn. Samples of ethylenediamine tetra-acetic acid (EDTA) plasma were frozen at – 20°C for analysis of fibrinogen and other immunologic parameters namely IgG Rheumatoid factors & IgM Rheumatoid factors, Interleukin 1 (IL-1), and Tumor necrosis factor.

Assessment of Immunological Parameters: The storage time of the frozen samples varied from approximately 1 to 3 months. All samples were stored under conditions considered to be appropriate for the subsequent measurement. Rheumatoid factor test (RA test) was carried out primarily qualitatively by slide method based on the principle of agglutination to detect presence or absence of RA factor using RHELAX RF reagent (Tulip diagnostic Ltd., Gitanjoli, Tulip Block, Bambolin, P.O. Goa-403202, INDIA). The reagent was standardized to detect ~ 10 IU/ml of RF or more. The Result was published as either RA test positive or negative on the basis of agglutination. Serum level of C - reactive protein (CRP) was measured by latex agglutination slide test method for qualitative and semi-quantitative determination of CRP of non-diluted serum using the reagent Humatex CRP (Human Gesellschaftfur Biochemica and Diagnostic gbH, Max-Planck-Ring-21, D-65205 wiesbaden,

Germany). The sensitivity was standardized to detect CRP concentration in non-diluted serum samples of approximately 6mg/L or higher.

Serum level of IgG rheumatoid factor (IgGRF) and IgM rheumatoid factor (IgMRF) were measured by Immunometric Enzyme Immunoassays for the quantitative measurement of IgGRF & IgMRF in serum using commercial kit (ORGen Te Diagnostika GmbH, Kupferbergterrasse 17-19, D-55116 Mainz, Germany). The sensitivity (the lower detection limits)for IgGRF, IgMRF were 1.0 U/ml. The normal reference values were <20 U/ml for both IgG RF and IgM RF. Immunometric assays (EIA) were used for quantification of interleukin 1a (IL-1a) and tumor necrosis factor alpha (TNF-a) by means of commercially available immunometric (EIA) Kit (cayman chemical company, 1180 East ELLS worth Road, Ann arbor, Michigan, 48108, USA). IL – 1-a (human) EIA Kit, Catalog No- 583311 for IL-1-a and TNF-a (human) EIA Kit. Catalog No-889201 for TNF-a – were used. The sensitivity for IL-1-a and TNF-a were same for their respective kit. The minimum detectable concentration was 1.5 pg/ml after 2 hours development period and 3.0 pg/ml after a 30 minute development period.

Hematological Biochemical Analysis: Hemoglobin (Hb), platelet count, total white blood cell count (WBC), westengren erythrocyte sedimentation rate in 1st hour, C-reactive protein (CRP), Blood urea, serum createnin, serum bilirubin, Aspertated-amino-transferase (AST), Alanin-amino-transferase (ALT), Blood sugar & serum proteins, and serum lipid profiles were analyzed at the laboratory affiliated to the respective Rheumatology centers. Hemoglobin level was measured by photometric colorimetric test method and the normal reference values were 14-18 gm/dl for males and 12-16 gm/dl for female. Total platelet count and white blood cell (WBC) count were measured at the routine laboratory of rheumatology centers and the normal reference values for platelete count was 250-400 x 10^9/L and white blood cell count was 4.0 – 11.0 x 10^3/L. Erythrocyte sedimentation rate was measured by westengren method and the normal reference values for male was 0-15mm and for female was 0-20mm in 1st hour. Serum total protein was measured by photometric colorimetric test- Biuret method using the reagent from manufacture (Crescent diagnostics, P.O. Box 2196 Jeddah 21451. MUSLCO SJ- Saudi Arabia) and the normal reference Values for adults was 6.6 to 8.7 g/L.Serum Albumin and globulin level were measured by colorimetric method using the reagent from the manufacturer(Crescent diagnostics, P.O. Box 2196 Jeddah 21451. MUSLCO SJ- Saudi Arabia). The normal reference value for serum albumin was 3.8-5.1g/L and for serum globulin was 2.9-4.3g/L. Serum level of fibrinogen was measured by direct

Table 4: Immunological and radiological indices of disease activity at baseline and months1, 3 and 6 . Data are presented as mean (SD).

	Modified diet + MTX (VD group)				Normal diet + MTX (ND group)				P values in between VD and ND		
	Base line N=40	Month 1 N=40	Month 3 N=35	Month 6 N=33	Base line N=40	Month 1 N=40	Month 3 N=37	Month 6 N=35	Month 1	Month 3	Month 6
CRP Mg l	40.16 (6.44)	31.24 (6.53) p<0.05	24.42 (6.76) P<0.05	15.38 (2.13) P<0.05	42.32 (7.20)	35.64 (5.57) P<0.05	30.86 (6.97) P<0.05	17.94 (2.12) P<0.05	P<0.05	P<0.05	P<0.05
IgG RF (Unit/ml)	74.75 (2.98)	64.96 (3.87) P<0.05	35.67 (4.72) P<0.05	26.25 (4.60) P<0.05	76.03 (6.06)	70.57 (6.90) P<0.05	40.32 (6.34) P<0.05	32.37 (5.72) P<0.05	P<0.05	P<0.05	P<0.05
IgM RF (Unit/ml)	78.31 (6.91)	69.56 (8.07) P<0.05	32.96 (3.94) P<0.05	28.75 (7.34) P<0.05	79.52 (8.68)	74.32 (9.27) P<0.05	43.08 (5.58) P<0.05	33.50 (11.45) P<0.05	P<0.05	P<0.05	P<0.05
1L-1 beta (pg/ml)	34.15 (2.92)		23.49 (1.93) P<0.05	18.78 (1.70) P<0.05	34.19 (2.90)		24.55 (1.73) P<0.05	20.80 (1.57) P<0.05		P<0.05	P<0.05
TNF alpha (pg/ml)	27.94 (4.15)		28.72 (4.28) P<0.05	27.37 (4.44) P=0.05	27.99 (3.60)		27.30 (4.10) P>0.05	27.78 (3.45) P>0.05		P>0.05	P>0.05
Radiographic erosion Score	9.50 (2.93)		10.56 (2.96) P<0.05	10.72 (3.06) P<0.05	10.75 (5.11)		12.11 (4.81) P<0.05	12.68 (4.88) P<0.05		P>0.05	P=0.05
Radiographic narrowing score	6.32 (1.97)		7.36 (2.06) P<0.05	7.37 (2.05) P<0.05	7.05 (3.48)		8.20 (3.47) P<0.05	8.42 (3.54) P<0.05		P>0.05	P>0.05

P values refer to level of significance to difference between modified diet & normal diet groups for changes from baseline to months 1, 3 & 6. Difference between groups were analyzed by student's t test for independent samples except for CRP evaluated by Mann-whitney U test; within group differences at months 1,3 & 6 compared with baseline, were evaluated by student's t test for paired samples except for CRP which was evaluated by wilcoxon signed ranks test.

ELISA where plasma fibrinogen protein was as test antigen using standard reagent Fibrinogen EIA (Paired antibodies) from the manufacturer (Affinity Biologicals Inc, Quadratech Ltd, P.O. Box – 167, Epsom, Surrey KT 18, 7YL, UK) for detection of fibrinogen in human plasma. The normal reference value was less then 3g/L.The serum bilirubin level was measured by Colorimetric method using the reagent from the manufacturers (Bicon ® Diagnostik, Hecke 8, D-34516 Marienhagen Germany), the normal reference value for adult was upto 17.1µmol/L. Serum aspertate-amino-transferase (AST) and alanine-amino-transferase (ALT) were measured by colorimetric determination according to the Reitman and Frankel method using the standard reagent by the manufacturer (Crescent diagnostics, P.O. Box 2196 Jeddah 21451. MUSLCO SJ- Saudi Arabia) and normal reference values for AST was <40 units/ml and ALT was <45 units/ml.Serum levels of urea was measured by urease-Berthelot colorimetric

method using the standard reagents from manufacturers (Bicon ® DiagnostiK, Hecke 8, D-34516 Marienhagen Germany) and the normal reference value was 15 to 45mg/dL. Serum createin level was measured by Jaffe method of creatinine Kinetic test without deproteinizations using the reagents from Spinreact S.A., etra santa coloma, 7E-17176 SANT Esteve DE BAS (GI) Espana and the normal reference value was 0.3-1.4 mg/dL. Plasma sugar level was measured by enzymatic colorimetric test without deproteinisation (SOD-PAP method) method using the standard reagent Glucose Liquicolor from the menofacturer (Human Gesellschatt fur Biochemica and Diagnostica mbH, Max-planck-Ring-21 – D-65205 Wiesbaden – Germany). The normal plasma (fasting) value was 4.2 – 6.4 m mol/L..Serum cholesterol and Triglycerides were measured by enzymatic colorimetric test method (CHOD-PAP for s. cholesterol GPO-PAP for S. Triglycerides) using the standard reagent from the menofacturer (Bicon ® DiagnostiK Hecke 8, D-34516 Marienhogen, Germany). The following reference values for serum cholesterol were recommended for the recognition of hyper-cholesterolemia; suspected above- 5.3 m.mol/L and increased risk above 6.7 m.mol/L.

Table 5 : Biochemical, hematological & nutritional parameters at base line and month 3 and 6; data are presented as mean (SD)

	Modified diet + MTX (VD group)			Normal diet + MTX (ND group)			Healthy control group			P values in between VD & control		P values in between VD & ND	
	Base line N=40	Month 3 N=35	Month 6 N=33	Base line N=40	Month 3 N=37	Month 6 N=35	Base line N=20	Month 3 N=20	month 6 N=20	month 3	month 6	Month 3	Month 6
BMI Wt in kg/height^2 in meter	24.75 (2.45)	24.65 (2.51) P<0.05	24.29 (2.51) P<0.05	25.27 (2.71)	25.32 (3.07) P>0.05	25.24 (3.05) P>0.05	25.47 (1.80)	25.30 (1.83) P<0.05	24.94 (1.73) P<0.05	P>0.05	P>0.05	P>0.05	P>0.05
Hb(gm/dl)	13.34 (1.59)	11.84 (1.42) P<0.05	10.84 (1.04) P<0.05	13.88 (1.08)	13.54 (0.97) P<0.05	13.39 (1.07) P<0.05	14.39 (1.30)	13.80 (1.04) P>0.05	13.92 (1.40) P>0.05	P<0.05	P<0.05	P<0.05	P<0.05
Total plasma protein(g/l)	7.881 (.242)	7.463 (.295) P<0.05	7.453 (1.50) P<0.05	7.860 (.151)	7.778 (.221) P>0.05	7.774 (2.29) P>0.05	7.525 (.116)	7.562 (.131) P>0.05	7.543 (1.22) P>0.05	P>0.05	P<0.05	P<0.05	P<0.05
Plasma cholesterol(mmol/l)	3.97 (0.17)	3.65 (0.44) P<0.05	3.19 (0.55) P<0.05	4.06 (0.67)	4.13 (0.71) P>0.05	3.83 (0.78) P>0.05	4.19 (0.45)	3.98 (0.30) P<0.05	3.95 (0.30) P<0.05	P<0.05	P<0.05	P<0.05	P<0.05
Plasma triglyceride(mmol/l)	0.88 (0.14)	0.76 (0.11) P<0.05	0.75 (0.11) P<0.05	0.85 (0.11)	0.82 (0.15) P>0.05	0.83 (0.19) P>0.05	0.82 (0.09)	0.75 (0.09) P<0.05	0.73 (0.11) P<0.05	P>0.05	P>0.05	P=0.05	P<0.05
Plasma fibrinogen g/l.	3.76 (0.41)	3.04 (0.21) P<0.05	3.10 (0.39) P<0.05	3.71 (0.17)	3.62 (0.22) P<0.055	3.73 (0.32) P>0.05	2.69 (0.15)	2.62 (0.23) P=0.05	2.62 (0.16) P=0.05	P<0.05	P<0.05	P<0.05	P<0.05
Plasma LDL(mmol)	2.38 (0.42)	2.11 (0.34) P<0.052	2.02 (0.35) P<0.05	2.42 (0.36)	2.40 (0.35) P>0.05	2.27 (0.45) P>0.05	2.57 (0.27)	2.45 (0.35) P>0.05	2.33 (0.49) P>0.05	P<0.05	P<0.05	P<0.05	P<0.05
Plasma HDL(mmol/l)	1.50 (0.22)	1.70 (0.22) P<0.05	1.90 (0.24) P<0.05	1.56 (0.13)	1.61 (0.18) P>0.05	1.61 (0.21) P>0.05	1.67 (0.18)	1.81 (0.23) P<0.05	1.79 (0.31) P<0.05	P>0.05	P>0.05	P=0.05	P<0.05

Difference between groups was analyzed by student's t test for independent samples. Significant change from baseline = P<0.05. Within group differences at months 1, 3 & 6 compared to baseline were evaluated by student's t test for paired samples.

Triglycerides values were 0.45 – 1.6 m.mol/L for women and 0.68 – 1.9 m.mol/L for men. Serum levels of HDL cholesterol and LDL cholesterol were measured by phosphotungstic, precipitation method using the standard reagent from the manufacturers (crescent diagnostics, P.O. Box 9939 Jeddah 21923 MUSLCO SJ-Saudi Arabia)

Normal reference value of HDL for male was 1.42 m mol/L & for female 1.68 m mol/L and, normal reference value for LDL was 1.6 – 3 m.mol/L.

Radiological Studies

Radiograph of the hands and wrists were obtained at base line and after 3 months, and again after 6 months of study. A standardized protocol for obtaining and scoring these radiographs was used in this study[35] and radiographs were evaluated by same radiologists in different stages of study.

Other investigations:_ECG of heart, ultra-sonography of kidneys and heart, X-ray of the chest, and routine examinations of urine for each patient were carried out at different stages of the study for evaluation of patient's conditions for the whole length of the study. Neurological examinations and ophthalmologic examinations of each patient were carried out by respective specialist at different stages of study period. These data were not presented herewith as the changes in the values of these parameters on the basis of dietary modifications were not discussed herewith.

Statistical Methods

Statistical analysis were done using SPSS for windows version 7.5.Differences between groups were evaluated by the Mann-Whitney U test for discrete variables, and student's test for independent samples were used for continuous variables. Within-group differences at 1,3 and 6 months when compared with baseline, were evaluated by Wilcoxon signed ranks test for discrete variables, while student's ttest for paired samples was performed for continuous variables.

Results

None of the baseline values were significantly different between the experimental group's namely vegetarian diet with MTX (VD group) and normal diet with MTX (ND) group. However, it is note worthy that the baseline values between experimental patient groups and healthy control group were not significantly different for serum lipid profiles but the differences were significant in all other parameters namely hematological, biochemical & also for the SF-36 health survey report. Comparison between VD group to healthy control group and; between VD group to ND group were also made for parameters which were seemed to be related to nutritional status. Among 100 subjects a total of 80 patients and 20 healthy persons were enrolled of whom 40 patients got MTX &

vegetarian diet supplemented with n-3 PUFAS & n-9 MUFAS (VD group), 40 patients got MTX & normal. Omnivorous diet (ND group), 20 healthy persons (control) got only modified vegetarian diet mentioned earlier. Twelve experimental patients were excluded from the final evaluation; of whom seven were in VD group and five were in ND group. The main reason of their dropout was the use of intra articuler injection with triamcinolone hexacetonide. The baseline values and the changes of all clinical, immunulogical, radiological, biochemical and hematological variables were summerises in table form -the comparison between groups have been presented here with.

Table-1 shows that the mean values of baseline characteristics of one group (back ground information) were almost close to the same value of other groups. Table-2 summerises both the baseline values and the changes of all clinical variables that took place during the study period in between two experimental groups namely VD group & ND group. At baseline no difference was seen between the VD group and the ND group. For the patients who were randomized to the vegetarian diet (VD) there was significant improvement in all clinical parameters in comparison to the improvement in normal diet (ND) group at the 6 months of study period .The difference of improvement towards significance in between two groups were seemed to be started from the 3 months of study for most of the parameters. Table-3 shows the quality of life reported by the patients using SF-36 health survey (version-2) which also favored the vegetarian (VD group). Table-4 represent the changes of immunological & radiological parameters of patients taking vegetarian diet (VD group) in comparison to the patients taking normal omnivorous diet(ND group).All the immunological parameters namely CRP, IgGRF. IgMRF, 1L-1 favored the vegetarian (VD group) highly significantly ($p<0.05$), but the TNF- alpha scarcely ($p>0.05$) favored the vegetarian at the 6 months of study.Though the radiological progression of disease activity was not stopped in both treatment groups, the radiographic erosion score. ($p=0.05$) and narrowing score ($p>0.05$) also favored the vegetarian group (VD) in comparison to normal omnivorous diet (ND)group.Table-5 shows the changes in laboratory biochemical, hematological & nutritional parameters between the experimental patient groups and control group. Among them all parameters significantly ($p<0.05$) favored the vegetarian (VD) group but hemoglobin level decreased significantly in VD group & also to healthy control group . Though the mean value of BMI (Body was index) significantly ($p< 0.05$) reduced in vegetarian (VD) diet group and healthy control group after 6 month of study but there was not statistically significant difference of BMI when compared to in between the groups – in between VD group & ND group ($p>0.05$) and in between ND group & control group ($p>0.05$).In comparison to base line; total plasma protein level at six months significantly reduced in VD group ($p<0.05$) but not in ND group ($p>0.05$) & control group ($p=0.05$). Fibrinogen level at 6

month also reduced highly significantly in VD group($p<0.05$) & significantly in control group ($p=0.05$) but not in ND group ($p>0.05$) when compared to corresponding base line values.Total plasma cholesterol level was highly significantly reduced in both VD($p<0.05$) &control ($p<0.05$) group but not in ND group ($p>0.05$) when compared to corresponding base line with values after 6 months.

Discussion

The study found vegetarian diet supplemented with monounsaturated fatty acid (MUFAS) and n-3 polyunsaturated fatty acids (PUFAS) had a suppressive effect on active & acute rheumatoid inflammation. A complete therapeutic evaluation will require larger number of patients to be followed up for a longer time and collaboration with other centers. When designing the study we made some assumption. Firstly we assumed that monounsaturated fatty acid & n-3 polyunsaturated fatty acids (PUFAS) suppress TNF alpha and 1L-1 beta production and action[17]Secondly low anti-oxidant intake results in enhanced cytokine production and effects It is noted that oxidant molecules up-regulate cytokine production. [17]Induction of nitric oxide and other oxidant molecules from phagocytic cells by 1L-1 ,1L-8 and TNF alpha- may promote this process and directly damage the host. Excessive, untimely,or prolonged production of cytokines have been associated with morbidity and mortality in a wide range of conditions including the rheumatoid arthritis[17].So we had supplied the diet to one group of experimental patient (VD group) to observe it's role on disease process and, to healthy control subjects to observe it's effects on nutritional status -- on the idea that this vegetarian diet supplemented with soybean oil and olive oil (dietary source of n-9 monounsaturated fatty acid & n-3 poly unsaturated fatty acids) may suppress TNF & 1L-1 production & action and on the other hand it may suppress the up-regulation or enhancement of cytokine production and action.As a vegetarian diet was enriched with n-3 PUFAS & n-9 PUFAS by supplementing soybean oil and olive oil to it – so we called it modified vegetarian diet.

A new dimension to the study of pro-inflammatory cytokines in chronic inflammatory diseases such as RA had been the finding of significantly increased expression of naturally occurring cytokine inhibitors .These include inhibitors which neutralize the cognate ligand either by binding directly such as soluble TNF receptors, or by blocking cell surface receptors such as the IL-1 receptor antagonist protein. [27] There are also a number of cytokines with known potent anti inflammatory properties, including IL-4, IL-10, IL-13 and TGF-beta. Of these IL-10 and TGF-beta are abundant in the joint. [28,29] These findings have raised some intriguing questions about the etiology and progression of the chronic inflammatory process. Clearly there is evidence for an attempt at. homeostasis.

Table 6: Description of Daily Dietary Allowance for Rheumatoid Arthritis Patients:

For male

Food Item	Raw Wt	Cooked Wt (gm)	Servings size	Calorie	Carbohydrate (gm)	Protein (gm)	Fat
Rice	200gm	600gm	9	690	158	12.8	0.8
Ata (wheat Flower)	100gm	135gm	4.5	307	62.5	10.9	1.5
Potato	100gm	106gm	1	97	22.6	1.6	0.6
Pulses	85gm	390gm	3	292	50.2	21.3	0.6
Leafy vegetables	150gm	160/95 gm	3	71	10.7	5.3	0.8
Other vegetables	150gm	173/120gm	3	66	12.3	2.9	0.6
Fruits	100gm	100gm	2	53	12.2	0.9	0.3
Milk	250ml	225ml	2	168	11.0	8.0	10.2
Sugar	30gm	30gm	-	120	30		
Fat/Oils(Soybean oil/olive oil)	70gm	70gm	-	630			70.00
				2499	**369.5**	**62.8**	**85.4**

For Female

Food Item	Raw Wt	Cooked Wt (gm)	Servings size	Calorie(ml)	Carbohydrate	Protein	Fat
Rice	150gm	450gm	7	518	118.5	9.6	0.6
Ata (wheat Flower)	90gm	135	4.5	307	62.5	10.9	1.5
Potato	75gm	80	1	73	17.0	1.2	0.4
Pulses	60gm	272	2	206	35.4	15.1	0.4
Leafy vegetables	150gm	160/95	3	71	10.7	5.3	0.8
Other vegetables	150gm	173/120	3	66	12.3	2.9	0.6
Fruits	100gm	100	2	53	12.2	0.9	0.3
Milk	250gm	225	2	168	11.0	8.0	10.2
Sugar	30gm	30		120	30gm		
Fat/Oils(Soybean oil/olive oil)	42gm	42gm		378			42.00
				1960	**309.6**	**53.9**	**56.8**

	K. cal	Carbohydrate	Protein	Fat
Small 1 (one) plate cooked Rice = 30gm÷3 =43.5gm (boiled rice(-)	150	34.4gm	2.8gm	0.2gm
Small 1/2 (half) plate cooked Rice = 65gm÷3 =22gm (boiled rice(-)	76	17.3gm	1.4gm	0.2gm

Vegetables which were made restricted: Beet, Cabbage, Lettuce, Pumpkin, Spinach, Tamarind leaves & tender, Colocasia, Yam, Brinjal, France beans, Ladies finger, peas and ground nuts.

Specifically, they raise the possibility that the chronic phase of the disease may persist not only for because of over production of pro-inflammatory cytokines but perhaps also because of inadequate anti- inflammatory or inhibitory responses. The balance of these factors is therefore thought to exert a profound influence on disease progression. It would appear that, during disease remission (either by drugs or by other means) the balance of cytokines and their cytokine inhibitors would favour the production of cytokine inhibitors, indicating that homeostasis has been restored at least to some extent[30] .To establish on assumption of homeostasis between pro-inflammatory cytokines and anti-inflammatory cytokines or to bring a balance between cytokines and cytokine inhibitors- we tried to formulate a vegetarian diet enriched with flavonoids with n-9 MUFAS & n-3 PUFAS –to down regulate IL-1 & TNF-alpha production & action , and to suppress or reduce the oxidant molecule up-regulating & enhancing cytokine production and action for the patient with active RA.

Fruits, vegetables & wild edible greens frequently eaten in rural Greece – contain very high quantities of flavonoids - which are generally considered one of the most important categories of anti-oxidants in the human diet [31]which may be intake to down-regulate cytokine production & action. Literatures suggested that n-3 poly unsaturated fatty acids such as alpha-linolenic acid present in soybean oil reduce the responsiveness to cytokines[32] and decreased IL – 1 beta level in plasma from RA[33] . We found in this study that there was difference of improvement for most of the parameters between diet (VD) group & ND group from baseline values to different stages of study- which had been started to become significant in diet group from early half of the study period and on wards. Literature supports that in patients with RA - the anti-inflammatory effect of Mediterranean diet supplementation and also fish oil supplementation was detectable after six weeks of study period[7].As both of the patient's (VD&ND group) received same drugs (MTX & NSAIDs), significant improvement in diet group (VD) for most of the parameters indicate that these improvement may be due to dietary modification – which findings were supported by many literature.[7,8,9,10,11,12,32].

An important component of the study design to promote optimal patient compliance with their vegetarian diet by this arrangement; the dietary prescription was designed as questionnaire to examine food choice mentioning the name and amount of locally available food items and each of the respective subjects were advised by the research rheumatologist and nutritionist to adopt the vegetarian type of diet. From previous literature on diet trials – we were impressed with how well the patients with RA seemed to had complied with the prescribed diets[13,11];and this study left us with the same impression .Important indicators were – the decrease in BMI, fall in serum cholesterol, triglyceride & total plasma proteins of the VD group and healthy control group[34].We included here with acute cases of active RA for this study but one literature expressed that there should be adequate control of disease and with newly diagnosed disease this would had been unacceptable [7], so we enrolled all acute cases

of RA with DAS 28 score more than 3.2 as with the EULAR criteria a DAS 28 more than 3.2 at the beginning of the study will be necessary, as a DAS 28 less than 2.0 indicates the absence of disease activity , and a change of 1.2 should be possible to be able to meet the (good) response criteria[22] .On the other hand a 1996 study suggested that early introduction of disease modifying anti-rheumatic drugs may be more beneficial than delayed introduction for patients with recently diagnosed RA and research was focused towards finding new , more effective drugs and treatment with combinations[32].From the overall findings from this study , we can say that by the first three months – the disease conditions may got an adequate control state with MTX & NSAIDs and , from the 3 months & onwards –further significant difference of improvement in VD group compared to ND group should be due to this dietary modification[7,10].A few patients had to take intra-articuler injections with triamcinolone hexaacetonide for relieve of pain – as this is the violations of the protocol- these patients were dropped from the study out.Literature suggested that weight loss had commonly been reported in response to dietary intervention for patients with RA [7,11,13] and we also found that BMI had been reduced in both the VD group and also to healthy control group.but in comparison between groups – there is no significant difference of change of values in between groups namely VD,ND & control. so this dietary modification has little significant influence on nutritional status of patient. In this study, we had found that this vegetarian diet group was favoured by most of the clinical and immunological parameters significantly- namely DAS 28 score ,the Ritchie's articuler index joint score, functional index of lee score , swollen joint count , tender joint count, ESR, pain VAS (0 – 10 mm), SF-36 health survey report, CRP (mg/L), total platelet count ($x10^{9}$) ,total WBC count($x\ 10^{3}$) , IgGRF (U/ml), IgMRF (u/ml), IL-1 beta (pg/ml) .Experimental study from Sweden[7] ,Norway[10]and Denmark[33] supported the findings of this study. Literature suggested that TNF-alpha level did not favour the vegetarian[10] – we also found that our modified vegetarian diet group favored by TNF-alpha scarcely ($p>0.05$).Hemoglobin concentration ,plasma cholesterol & triglycerid level and serum protein levels were reduced in VD group.These findings were also supported by the study carried out in Oslo, Norway[34] & also in Sweden[7].Though worsening of the erosion score & joint space narrowing score was observed in both groups-slower rate of radiographic progression was found in VD group compared with ND group. A study with combined therapy regimen for early rheumatoid arthritis supported these findings[32].In conclusion, the result of this intervention study indicate that a vegetarian diet supplemented with n-9 monounsaturated fatty acids and n-3 polyunsaturated fatty acids suppressed disease activity of patients with RA. Eating a modified vegetarian diet for six months- patients with active RA can obtain a better quality of life scored by version- 2 of the SF-36 Health survey.

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