ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 03 | March - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

A COMPARATIVE EVALUATION OF STATIN AND FENOFIBRATE (LIPID LOWERING DRUGS) ON THE INTERACTION OF PRO¬-INFLAMMATORY CYTOKINES AND VASOMOTOR INSTABILITY IN WOMEN WITH NATURAL AND SURGICAL MENOPAUSE.

Gynaecology

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ABSTRACT

Introduction: Evidence suggests that the decline in ovarian function with menopause is associated with spontaneous increase in proinflammatory cytokines, especially IL-1, IL-6 and TNF-α.

Objective: To evaluate the possible relationship of pro-inflammatory cytokines and vasomotor symptoms in menopausal women on lipid lowering drugs- statin and fenofibrate.**Materials &Method:** A prospective randomized clinical trial was carried out in the Dept. of Obstetrics and Gynaecology, I.P.G.M.E.R., Kolkata enrolling 100 women and dividing them into six groups. They were given either atorvastatin or fenofibrate or no drug. The main outcome measures were to estimate changes in cytokine levels and vasomotor symptoms and their correlation. **Results:** Although the cytokine levels (except TGF-β) & incidence of vasomotor symptoms increased with menopause, there was only some positive correlation between TNF-α with increasing symptom at three month postoperative period. With atorvastatin therapy, moderate positive correlation (r_{PB}>0.5) exists between decreasing levels of IL-1, IL-6 and TNF-α and decreasing vasomotor symptoms & strong negative correlation [r_{PB} = -0.79] in case of TGF-β in natural menopausal women. In surgical menopausal women treated with atorvastatin correlation exists between TNF-α and TGF-β levels and vasomotor symptoms.There was no correlation found between any of the cytokine level and vasomotor symptom in women given fenofibrate in natural menopausal group.but correlation found between TNF-α and TGF-β levels and vasomotor symptoms in the other group of subjects (for TNF-α r_{PB}=+0.35 and for TGF-β r_{PB}=−0.49).**Conclusion:** The levels of inflammatory cytokines IL-1, IL-6, TNF-α increased within three months of surgical menopause while the level of anti-inflammatory cytokine TGF-β decreased. Atorvastatin and fenofibrate- both are effective in alleviating vasomotor symptoms, though statistically insignificant. The levels of inflammatory cytokines, TNF-α and TGF-β are best correlated with vasomotor symptoms in menopausal women.

KEYWORDS

Atorvastatin and fenofibrate, pro-inflammatory cytokines,vasomotor symptoms.

INTRODUCTION

Menopause,the permanent cessation of ovarian function marks the end of reproductive capacity of women. Surgical menopause is distinct from natural menopause as the abrupt cut off of ovarian hormones causes the sudden onset of hot flashes and other menopause related symptoms. There is now a large body of evidence suggesting that the decline in ovarian function with menopause is associated with spontaneous increase in proinflammatory cytokines, especially IL-1, IL-6 and TNF-α[1]. Experimental and clinical studies strongly support a link between the increased state of proinflammatory cytokine activity and postmenopausal bone loss[2]. Estrogen deficiency has also been shown to enhance the responsiveness of cells toward some of these cytokines by upregulating cytokine receptor numbers and cofactors of cytokine action.

Hot flashes are the most common symptom of the climacteric; affects 3/4th of menopausal women and are one of the most common health problems in this demographic group. Hot flashes typically begin 1-2 years before menopause and commonly persist for 6 months to 5 years.Hormone therapy has long been recognised as the primary treatment for hot flashes and reduces their frequency in menopausal women by 70-90%[3]. This therapy however increases risk of stroke, thromboembolic events, heart disease and breast cancer. Given these growing concerns and subsequent risks, other nonhormonal pharmacologic alternatives are necessary for the long-term treatment of hot flashes.

Statins and fenofibrates are lipid lowering drugs that have been shown to have anti-inflammatory effects both in vitro and in vivo[4]. Statins and fibrates can exert antithrombotic and anti-inflammatory effects as early as after 3 days of therapy. Plasma markers of inflammation and homeostasis and monocyte release of proinflammatory cytokines like TNF-α, IL-1β, IL-6 and monocyte chemoattractant protein1 reduces with the above two groups of drugs[5].

In view of the emerging role of inflammation as an important component of the pathogenesis of menopause related disorders, it may be worthwhile to study the effects of statin and fenofibrate on proinflammatory cytokine activity and its relationship with vasomotor activity in females with natural and surgical menopause.

MATERIALS AND METHODS

A prospective randomized controlled trial was carried out in the Dept. of Obs and Gynae of I.P.G.M.E.R. and S.S.K.M Hospital, Kolkata for 18 months. Total 100 women were enrolled after taking proper written consent- 50 with natural menopause occurring within last 5 years and 50 women who were destined to have surgical menopause due to total hysterectomy with bilateral salpingo-oophorectomy (TH+BSO) for any benign uterine or ovarian disorder within a short time, were recruited for the study. Women with any organic disease like cardiovascular disease, cerebrovascular accident, epilepsy and diabetes mellitus with or without hypertension were excluded from the study. Each group of subjects were randomly divided into 3 subgroups. They were given Atorvastatin 10mg/day in Gr.A, Fenofibrate 200mg/day in Gr.B and no drug at all in Gr. C. The study subjects were sequentially monitored by questionnaires and by clinical examination at 0, 3, 6 months. For surgical menopausal women, 0 month was immediate preoperative period, 3 month and 6 months were postoperative visit after 3 month and 6 months of the operation. The laboratory investigations were carried out at 0, 3, 6 months as well.The investigations done were-Plasma Lipids(total cholesterol, triglyceride, HDL, VLDL, LDL), endocrine profile(FSH, LH, estradiol, progesterone),fasting blood sugar,neurotransmitters (serotonin, dopamine), proinflammatory cytokines (IL-1, IL-6, TNF-α) and TGF-β (anti-inflammatory cytokine).

Laboratory techniques used were-

-FSH, LH, Estradiol, Progesterone-- by commercial kits based on Sandwich ELISA; Dopamine, Serotonin by reverse phase high performance liquid chromatography with electrochemical detector; Lipid profile by commercial kit in an auto analyser; Proinflammatory

cytokines—by quantitative Sandwich ELISA.

The drugs were given from 0 month to 6 months continuously for natural menopausal women whereas in surgical menopausal women the drug was started at 3 months postoperative period and given for 3 months upto 6 months postoperative period.The subjects who have completed the study period were evaluated for the assessment of the interactions of proinflammatory cytokines with vasomotor instability following the drug treatment.

Statistical analysis was done by: For categorical variables- Mc Nemar's test 2-tailed p; For numerical variables- Student's paired 't' test where sample size was >30 and Wilcoxon's matched pair signed rank test where sample size was <30. Correlation coefficient - Point biserial correlation coefficient.

RESULTS

Total 92 patients completed the study as per strict follow up protocol. Among natural menopausal women (N=44) the most prevalent symptom was urogenital (70.45%) followed by vasomotor symptom (54.54%). In surgical menopausal women(N=48), prevalence of vasomotor symptom increased from 16.66% to 31.25% three months after surgery, which was statistically significant (P=0.039). In these women the mean level of FSH, LH, estradiol and progesterone changed significantly within this time period. Serotonin and dopamine level decreased. IL-1, IL-6, TNF-α levels increased significantly while TGF-β level decreased significantly at the same time.

Table 1. Comparison of the levels of various parameters in surgical menopausal women before (0 month) and 3 month after TH+BSO operation

	Parameters	0 month	3 month	*p value
Lipids	Total Cholesterol (mg/dl)	157.60 ± 41.91	147.70 ± 39.27	0.005
	Triglyceride (mg/dl)	148.93 ± 52.15	142.25 ± 42.79	0.106
	HDL (mg/dl)	37.04 ± 8.09	41.35 ± 7.81	<0.001
	VLDL (mg/dl)	29.68 ± 10.39	28.37 ± 8.48	0.124
	LDL (mg/dl)	95.18 ± 25.87	98.04 ± 25.27	0.186
Hormones	FSH (IU/L)	11.39 ± 3.95	40.48 ± 15.95	<0.001
	LH (U/L)	7.13 ± 2.17	26.13± 6.68	<0.001
	Estradiol (pg/ml)	143.33 ± 99.37	9.37 ± 2.17	<0.001
	Progesterone (ng/ml)	10.34 ± 6.58	0.78 ± 0.16	<0.001
Neurotransmitters	Serotonin (pmol/L)	332.20±33.64	285.85±42.76	<0.001
	Dopamine (pmol/L)	490.70±48.89	381.00±54.06	<0.001
Cytokines	IL-1 (pg/ml)	5.13 ± 4.13	49.61 ± 21.94	<0.001
	IL-6 (pg/ml)	15.91 ± 6.48	95.17 ± 34.66	<0.001
	TNF-α (pg/ml)	17.64 ± 8.46	135.74 ± 61.15	<0.001
	TGF-β (ng/ml)	60.36 ± 56.36	24.37 ± 4.90	<0.001

*by Student's paired t test
-values given are mean ± Standard Deviation

All parameters except neurotransmitters were measured in 92 subjects. Neurotransmitters were measured in total 20 subjects and the test done is Wilcoxon's matched pairs signed rank test.

Atorvastatin decreased the prevalence of vasomotor symptoms from 61.11% to 33.33% in natural menopausal women and from 30% to 15% in surgical menopausal women. There was improvement in the symptoms with fenofibrate, too, but the results didn't reach statistical significance (Mc. Nemar's test 2 tailed P value> 0.05). Both natural and surgical menopausal women who were given no drug therapy had worsening of symptom during the study period.

Cytokine levels did not change significantly in women given no drug therapy during the study period.In natural menopausal women given atorvastatin, the initial (0 month) mean level of IL-1, IL-6, TNF-α TGF-β was 27.05 pg/ml, 62.02 pg/ml, 213.26 pg/ml and 19.64 ng/ml respectively. The levels changed to 24.02 pg/ml, 58.73 pg/ml, 187.85 pg/ml and 22.86 ng/ml respectively. By Wilcoxon's matched pairs signed rank test the p values of change of IL-1 is 0.5277, IL-6 is 0.527, TNF-α is 0.248 and TGF-β is 0.0777. These showed **statistically non-significant change in cytokine level with atorvastatin in natural menopausal women.In surgical menopausal women, the levels of TNF- α decreased significantly**(p=0.006) **and TGF- β increased significantly** (p=0.043) **with atorvastatin.**Fenofibrate reduced the levels of IL-1, IL-6, TNF-α in both natural and surgical menopausal women, but only the change in the level of TNF-α (p=0.002) in surgical menopausal group was statistically significant.

Table2. Comparison of statistical significance of change of cytokine levels in natural and surgical menopausal women with or without drug treatment

Cytokines	*p value					
	No drug		Atorvastatin		Fenofibrate	
	Natural Menopause	Surgical Menopause	Natural Menopause	Surgical Menopause	Natural Menopause	Surgical Menopause
IL-1	0.888	0.441	0.527	0.167	0.327	0.334
IL-6	0.068	0.109	0.527	0.191	0.325	0.375
TNF-α	0.575	0.085	0.248	0.006	0.144	0.002
TGF-β	0.779	0.952	0.077	0.043	0.810	0.243

* by Wilcoxon's matched pairs signed rank test.

In natural menopausal women treated with atorvastatin, there was positive correlation between presence or absence of vasomotor symptom and IL-1(r_{PB}= +0.52), IL-6 (r_{PB}= +0.57), TNF-α (r_{PB}= +0.61) at 6 months and negative correlation with TGF-β level(r_{PB}= -0.79) at 6 months. In surgical menopausal women, both the drug groups showed correlation between the levels of TNF-α and TGF-β with vasomotor symptoms at 6 month time.

Table3. Point biserial correlation coefficient (r_{PB} value)of presence or absence of vasomotor symptoms vis-a-vis individual cytokine levels in natural and surgical menopausal women after drug treatment

A.Natural Menopausal

Variable	Atorvastatin		Fenofibrate	
	r_{PB}	p value	r_{PB}	p value of correlation coefficient
IL-1 time-0 mth	+ 0.04	0.875	– 0.2	0.430
IL-1 time-6 mth	**+ 0.52**	**0.028**	– 0.13	0.597
IL-6 time-0 mth	– 0.37	0.136	+ 0.16	0.531
IL-6 time-6 mth	**+ 0.57**	**0.013**	– 0.18	0.476
TNF-α time- 0 mth	+ 0.05	0.844	– 0.27	0.283
TNF-α time-6 mth	**+ 0.61**	**0.007**	+ 0.19	0.441
TGF-β time- 0 mth	**– 0.62**	**0.006**	+ 0.01	0.961
TGFβ time -6 mth	**– 0.79**	**0.001**	– 0.20	0.419

B. Surgical Menopausal

Variable	Atorvastatin		Fenofibrate	
	r_{PB}	p value	r_{PB}	p value of correlation coefficient
IL-1 time-3 mth	-0.06	0.797	-0.19	0.411
IL-1 time-6 mth	+0.13	0.581	-0.06	0.812
IL-6 time-3 mth	+0.06	0.774	-0.01	0.976
IL-6 time-6 mth	+0.28	0.211	+0.07	0.823
TNF-α time- 3 mth	+0.61	0.002	-0.23	0.389
TNF-α time-6 mth	+0.43	0.047	+0.35	0.119
TGF-β time- 3 mth	-0.29	0.195	-0.12	0.609
TGFβ time -6 mth	-0.51	0.014	-0.49	0.022

(r_{PB} value if < 0.3 suggests poor/no correlation; 0.3-0.5 =fair/some correlation; 0.5-0.7=moderate correlation; ≥0.7=strong correlation. For any r_{PB} value, if p value is ≤ 0.05, then the correlation is sufficiently strong and probably it is true for the underlying population.)

DISCUSSION

Spontaneous increase in the expression and secretion of the proinflammatory cytokines IL-1, IL-6, TNF-α with estrogen deficiency were noted several years ago in ex vivo cultures of circulatory monocytes, bone marrow macrophages and osteoblasts[6] Using extremely sensitive techniques, such as IL-6 promoter leuciferase constructs, increases in IL-6 promoter activity have been observed in the spleens of ovariectomized transgenic mice harbouring

these [7] Neverthless, the relation between estrogen and these cytokines is far from being that simple. In fact, the existing literature is replete with seemingly contradictory data, because an equally large number of studies have failed to demonstrate any effects of estrogen on the expression or concentrations of these cytokines. Even more puzzling, some researchers have even reported biphasic or frankly stimulatory effects of estrogen on the release of these cytokines from monocytes/macrophages and fibroblast like synoviocytes[8]. They raise the hypothesis that there may be several distinct pathways by which estrogen may affect cytokine gene expression, eliciting either net increase or decrease in cytokine production, depending on the activation of these pathways in the individual cellular context.

In our study, the levels of IL-1,IL-6,TNF-α increased significantly after TAH+BSO within three months time.Change in cytokine level corroborate with finding got by Cantatore FP, Loverro G et al[9], who treated women after total hysterectomy and bilateral salpingooo phorectomy by either estrogen, estrogen and progesterone or no drug and showed significant increase in the levels of IL-1, IL-6 in women given no drug. Women who received hormone treatment showed no significant change in the cytonkine levels.

Atorvastatin has anti-inflammatory effect in its therapeutic doses and reduces the inflammatory cytokine levels. H. Hsu, P. Wang et al showed in their study[10] that changes in IL-1β, IL-6, TNF-α and soluble endothelin (ET-1) showed trends towards a progressive decline after atorvastatin therapy, but none of the cytokines was reduced significantly. Similarly,Madej A, Okopien B showed that[11] fenofibrate is effective in decreasing cytokine levels in hyperlipidemic subjects in whom initial cytokine level was greater than healthy subjects. The levels of proinflammatory cytokines IL-1, IL-6, TNF-α decreased by either drug therapy in our study, while the serum level of anti-inflammatory cytokine TGF-β increased. The change in the levels of TNF-α and TGF-β was statistically significant in surgical menopausal women.

Although the levels of cytokines (except TGF-β) and incidence of vasomotor symptoms increased with menopause, only the level of TNF-α was positively correlated (point-biserial correlation coefficient>r_{PB} value=+0.44) with increasing symptoms. TGF-β level showed very weak correlation (r_{PB} value=+0.33). Atorvastatin and fenofibrate showed some effectivity in reducing vasomotor symptoms, but not upto statistical significance.. The changes in the levels of TNF-α and TGF-β could be correlated with the occurrence of vasomotor symptoms in almost all subgroup of patients.

CONCLUSION

Estrogen deficiency following menopause is associated with increasing circulatory proinflammatory cytokines & vasomotor symptoms. Compounds that directly interact with cytokine activity may be more effective than hormone replacement therapy in neutralising menopause related health disorders.

Two lipid lowering agents, atorvastatin and fenofibrate are claimed to have anti-inflammatory properties and effective in alleviating vasomotor symptoms. The levels of TNF-α and TGF-β are best correlated with vasomotor symptoms in menopausal women on these drugs.Therapeutic implication of the above drugs as an alternative to hazardous hormone replacement therapy is yet to be settled down by large multicentric randomized controlled trials.

Author's declaration:

There was no financial or other Competing Interests. The study was approved by the Ethics Committee of the institute. Informed consentwas obtained from the subjects involved i n the study.

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