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Erratum

Significant association of TREM-1 with HMGB1, TLRs and RAGE in the pathogenesis of insulin resistance in obese diabetic populations: Am J Transl Res. 2017; 9(7): 3224-3244

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In this article published in AJTR0048994, in further review of data during continuation of study, we realized certain patients didn't meet inclusion criteria. The reanalysis of the new dataset however didn't change our final conclusion.

The data was reanalysed per IRB guidelines after removing three subjects who either had BMI > 65 or BMI < 40 (Even though they undergone bariatric surgery). One control subject was also needed to be removed from the study. We included total subjects (41 study subjects and 4 control subjects) in the study (**Table 1**). Biochemical profile and fatty liver grading is performed (**Tables 2**-**4**). A possible role in the underlying pathophysiology of obesity and associated co-morbidities. We examined the mRNA expression by RT-PCR and protein expression by Western blotting and immunofluorescence for TREM-1, TREM-2, DAP-12, HMGB-1, RAGE, TLR-4 and TLR-2 in omentum, subcutaneous and liver biopsy tissues of obese diabetic (n = 18) and non-diabetic subjects (n = 23) and compared with the non-obese non-diabetic controls (n = 4). There was a significantly increased expression of TREM-1, DAP-12, HMGB-1, RAGE, TLR-4 and TLR-2 and decreased expression of TREM-2 in the omentum, subcutaneous and liver biopsy of obese diabetic subjects compared to obese non-diabetics and the non-obese population (**Table 4**). Overall, obese diabetic subjects had high expression of TREM-1 in association with HMGB1 (100% vs 58.3%, P = 0.006), RAGE (77.3% vs 41.7%, P = 0.045), TLR4 (100% vs 58.3%, P = 0.006), and TLR2 (100% vs 50%, P = 0.002) in liver biopsy samples in comparison to obese non-diabetic subjects. Obese diabetics have significantly increased TREM-1, HMGB1, RAGE, and TLRs compared to obese non-diabetics (**Table 5**). Our findings suggest a potential pathophysiological role of TREM-1 in conjunction with HMGB1 and inflammatory cell receptors (RAGE, TLR-4 and TLR-2) in obesity-induced insulin resistance.

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Table 1. Demographics and co-morbid conditions of study population

Clinical data	Non-obese (4)	Obese non-diabetics (23)	Obese diabetics (18)	Correlation (R); *P* value
Demographics				
Gender (Male/Female)	0/4	2/21	5/13	
Age (year)	46.25 ± 16.68	39.82 ± 10.08	49.55 ± 10.89	
BMI	26.17 ± 1.82	46.60 ± 6.2	47.82 ± 8.08	
Height (feet' inches")	5.72 ± 0.09	5.41 ± 0.26	5.60 ± 0.28	NS
Weight (pounds)	151.75 ± 9.94	282.78 ± 57.93	299.13 ± 58.56	
Co-morbid conditions				
Hypertension	1 (25%)	11 (47.8%)	16 (88.9%)**	R = 0.430; P = 0.006
Hyperlipidemia	-	10 (43.5%)	10 (55.6%)	NS
Sleep apnea	-	5 (21.7%)	11 (61.1%)*	R = 0.401; P = 0.012

Demographics and co-morbidities were compared between obese non-diabetics and obese diabetics using student t-test for continuous variables and Fisher's exact test or Pearson's χ^2 for categorical variables. Not significant (NS), data for age, body mass index (BMI), height and weight are presented as mean values ± SD number (percentage) of patients. Since, the participation of non-obese subjects were limited in this study, we have used the tissue biopsies of non-obese subjects from our previous work [14] for this study. Data in co-morbid condition show number of subjects (%) in that group. *P < 0.05 and **P < 0.01.

Table 2. Biochemical profile of obese patient population

Biochemical Profile	Obese non-diabetics (23)		Obese diabetics (18)		*P* value
	Biochemical levels	N > normal values	Biochemical levels	N > normal values	
Cholesterol (mg/dl), >200	170.71 ± 37.00	6	161.44 ± 34.27	3	NS
Triglycerides (mg/dl), >149	149.91 ± 59.64	9	199.5 ± 126.36	14	NS
FFA (μM/ml), > 0.65	0.85 ± 0.29	16	1.37 ± 0.52***	16	P = 0.0002
VLDL (mg/dl), > 30	30.73 ± 12.08	8	38.83 ± 20.33	12	NS
HDL (mg/dl), < 40	43.21 ± 9.19	11	47.274 ± 17.42	11	NS
LDL (mg/dl), > 99	95.686 ± 31.91*	9	74.16 ± 33.28	6	P = 0.04
Cholesterol:HDL >4.4	4.06 ± 1.12	6	3.71 ± 1.36	7	NS
LDL:HDL >3.2	2.29 ± 0.90	4	1.93 ±1.15	3	NS
HbA1c (%), >6	5.54 ± 0.56	7	7.32 ± 0.95****	18	P < 0.0001
Glucose (mg/dl) > 100	98.43 ± 15.89	11	154.55 ± 45.33****	18	P < 0.0001
Insulin (μIU/mL) > 8.4	13.98 ± 6.11	15	27.13 ± 13.04****	18	P = 0.0001
HOMA-IR, >2	3.54 ± 1.93	12	10.57 ± 6.85****	18	P < 0.0001
HOMA-β%, >100	159.52 ± 91.02	21	126.07 ± 69.60	11	NS

Biochemical profile comparison was done between obese non-diabetics and obese diabetics subjects using student t-test for continuous variables. All data are presented as mean values ± SD, (normal physiological levels), *p* values for significance. Not significant (NS), Free fatty acids (FFA), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), glycosylated hemoglobin (HbA1c), homeostatic model assessment (HOMA)-Insulin resistance (IR). *P < 0.05, ***P < 0.001 and ****P < 0.0001.

Table 3. Grading of Fatty liver in obese subjects

Fatty liver grading	Obese (41)		
	Obese non-diabetics (23)	Obese diabetics (18)	Correlation (R); *P* value
(i) Inflammation	11 (47.8%)	11 (61.1%)	NS
No inflammation	-	-	
Minimal	8 (34.7%)	4 (22.2%)	
Mild	2 (8.6%)	7 (38.8%)	
Moderate	1 (4.3%)	-	
Severe	-	-	
(ii) Hepatosteatosis	10 (43.5%)	15 (83.3%)**	R = 0.405; P = 0.010
0%	-	-	
0-33%	7 (30.4%)	6 (33.3%)	
33-66%	3 (13%)	3 (16.6%)	
66-100%	-	6 (33.3%)	
(iii) Fibrosis	5 (21.7%)	7 (38.9%)	NS
Portal fibrosis	5 (21.7%)	5 (27.7%)	
Periportal fibrosis	-	1 (5.5%)	
Septal fibrosis	-	-	
Cirrhosis	-	1 (5.5%)	

Fatty liver grading was compared between obese non-diabetics and obese diabetics using Fisher's exact test or Pearson's χ^2 for categorical variables. Inflammation in liver biopsy was categorized with no inflammation, minimal, mild, moderate and severe inflammation; hepatosteatosis was categorized as 0%, 0-33%, 33-66% and 66-100%; and fibrosis was categorized as portal, periportal and septal fibrosis and cirrhosis for classification. Data show number of subjects in respective group (% subjects). **P < 0.01.

Table 4. Expression of TREM-1, TREM-2, DAP-12, TLR2, TLR4, HMGB-1 and RAGE in obese subjects compared to non-obese subjects

Target genes expression in obese biopsy samples (41)		Obese non-diabetics (23)	Obese diabetics (18)	Correlation (R); *P* value
Omentum				
TREM-1	39 (95.1%)	21 (91.3%)	18 (100%)	NS
TREM-2	36 (87.8%)	18 (78.3%)	18 (100%)	NS
DAP-12	37 (90.2%)	19 (82.6%)	18 (100%)	NS
HMGB1	35 (85.3%)	17 (73.9%)	18 (100%)*	R = 0.366; P = 0.022
RAGE	30 (73.1%)	15 (65.2%)	15 (83.3%)	NS
TLR-4	40 (97.5%)	22 (95.7%)	18 (100%)	NS
TLR-2	37 (90.2%)	19 (82.6%)	18 (100%)	NS
Subcutaneous				
TREM-1	26 (63.4%)	13 (56.5%)	13 (72.2%)	NS
TREM-2	24 (58.5%)	11 (47.8%)	13 (72.2%)*	NS
DAP-12	22 (53.6%)	9 (39.1%)	13 (72.2%)*	R = 0.329; P = 0.036
HMGB1	21 (51.2%)	9 (39.1%)	12 (66.7%)*	NS
RAGE	17 (41.4%)	8 (34.8%)	9 (50%)	NS
TLR-4	26 (63.4%)	13 (56.5%)	13 (72.2%)	NS
TLR-2	21 (51.2%)	9 (39.1%)	12 (66.7%)*	NS
Liver				
TREM-1	30 (73.1%)	12 (52.2%)	18 (100%)****	R = 0.536; P < 0.0001
TREM-2	27 (65.8%)	13 (56.5%)	14 (77.8%)	NS
DAP-12	30 (73.1%)	12 (52.2%)	18 (100%)****	R = 0.536; P < 0.0001
HMGB1	25 (60.9%)	7 (30.4%)	18 (100%)****	R = 0.708; P < 0.0001

RAGE	18 (43.9%)	5 (21.7%)	13 (72.2%)**	R = 0.505; P = 0.002
TLR-4	25 (60.9%)	7 (30.4%)	18 (100%)****	R = 0.708; P < 0.0001
TLR-2	24 (58.5%)	6 (26.1%)	18 (100%)****	R = 0.745; P < 0.0001

Expression of TREM-1, TREM-2, DAP-12, TLR2, TLR4, HMGB-1 and RAGE in obese subjects compared to non-obese subjects. Higher number of subjects with increased expression of TREM-1, DAP-12, TLR2, TLR4, HMGB-1 and RAGE and down regulation of TREM-2 were analyzed between obese non-diabetics and obese diabetics using Fisher's exact test or Pearson's χ^2 for categorical variables. Data show number of subjects having higher values of these compared to control non-obese subjects. Values show number of subjects (% subjects of total), not significant (NS). *P < 0.05, **P < 0.01 and ****P < 0.0001.

Table 5. Correlation between TREM-1 with DAP-12, TLR2, TLR4, HMGB-1 and RAGE in omentum, subcutaneous and liver tissues of study subjects

Target genes correlation	Obese non-diabetics (OND)	Obese diabetics (OD)	Correlation (R); *P* value
Increased TREM-1 (OND-21/23; OD-18/18) association with other genes in omentum biopsy samples			
DAP-12	19/21 (90.5%)	18/18 (100%)	NS
HMGB1	17/21(81.0%)	18/18 (100%)	NS
RAGE	15/21 (71.4%)	18/18 (100%)	NS
TLR-4	21/21 (100%)	18/18 (100%)	NS
TLR-2	19/21 (90.5%)	18/18 (100%)	NS
Increased TREM-1 (OND-13/23; OD-13/18) association with other genes in subcutaneous biopsy samples			
DAP-12	9/13 (69.2%)	13/13 (100%)*	R = 0.426; P = 0.048
HMGB1	9/13 (69.2%)	12/13 (92.3%)	NS
RAGE	8/13 (61.5%)	9/13 (69.2%)	NS
TLR-4	13/13 (100%)	13/13 (100%)	NS
TLR-2	9/13 (69.2%)	12/13 (92.3%)	NS
Increased TREM-1 (OND-12/23; OD-18/18) association with other genes in liver samples			
DAP-12	12/12 (100%)	18/18 (100%)	NS
HMGB1	7/12 (58.3%)	18/18 (100%)**	R = 0.548; P = 0.006
RAGE	5/12 (41.7%)	13/18 (72.2%)*	R = 0.356; P = 0.045
TLR-4	7/12 (58.3%)	18/18 (100%)**	R = 0.548; P = 0.006
TLR-2	6/12 (50%)	18/18 (100%)***	R = 0.612; P = 0.002

Subject's categorical variables with correlation between TREM-1 and DAP-12, TLR2, TLR4, HMGB-1 and RAGE were analyzed among obese non-diabetics and obese diabetics using Fisher's exact test or Pearson's χ^2 test. Data show number of subjects having higher values of these compared to control non-obese subjects. Values show number of subjects (% subjects of total), not significant (NS). *P < 0.05, **P < 0.01 and ***P < 0.001.