Novel non-invasive diagnostic options for endometriosis - based on glycome analysis

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Abstract

There is little published on endometriosis and glycosylation, and most of the studies are conducted with tissue or peritoneal fluid samples, collected by invasive means. An Iraqi study draws attention to the importance of serum sialylation, which is dramatically changed in endometriosis patients after zoladex therapy (1), indicating that changes in serum sialylation may be a new biomarker of the disease. While glycosylation of urine in endometriosis has not been studied so far, in a study of endometrial cancer, the urinary levels of two glycoproteins were significantly increased in the patients compared to the control group. This is a prospective study, serum and urine samples were collected for glycan analysis in women with and without endometriosis, as diagnosed at laparoscopy (MFC). This study was approved by the Research and Ethics Committee of the National Maternity Hospital, Dublin (EC19.2018). Additional endometriosis serum cohort (IND) was analyzed for validation of these results. *N*-glycome from all serum and urine glycoproteins and serum IgG were analyzed using hydrophilic interaction liquid chromatography-ultra performance liquid chromatography (HILIC-UPLC) and mass spectrometry.

Results

N-glycome of serum IgG in endometriosis

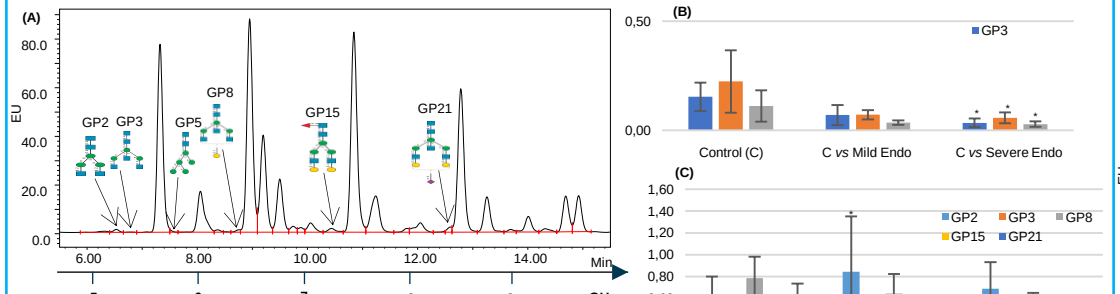
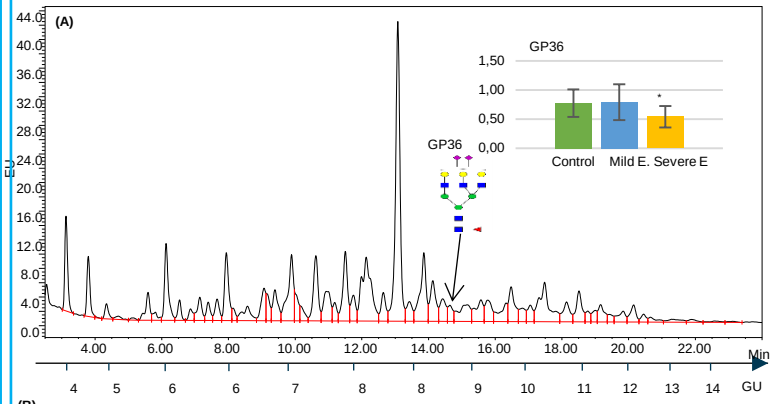


Figure 2. (A) Representative HILIC-UPLC chromatogram represents the IgG *N*-glycome profile of a control (without endometriosis) sample and highlighted are the altered GPs in endometriosis. Main glycans are pictured based on Pučić et al (3). The bar charts of **(B)** show the significant changes in case of MFC cohort, meanwhile the figure **(C)** illustrates the findings of IND study group. The significant alterations between the mild and severe endometriosis vs control group were plotted and marked with an asterisk. *= p<0.05; **= p<0.01.

Whole urine *N*-glycome in endometriosis



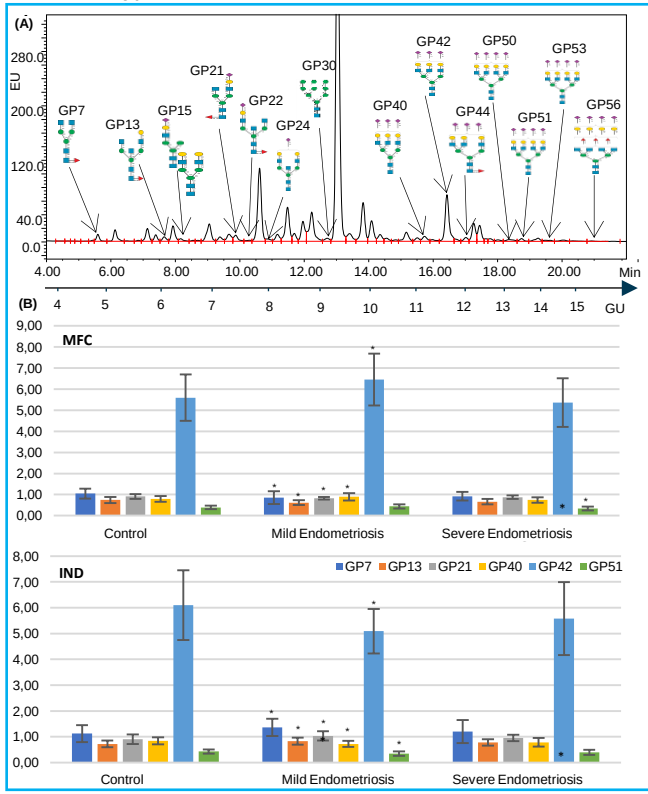
(B)

GP	Structure	GP	Structure	GP	Structure
GP 1	A1	GP 21	FA2G1GalNAc[SO4-2]1S(3)1	GP 31	A2G2S(6)2
GP 2	M4		M7		FA2G1GalNAc1S(6)2
GP 3			M4A1G1S(6)1		FA2G2S(3,6)2
GP 4	FA1		A2G1GalNAc[SO4-2]1S(6)1	GP 32	FA2F1G2(Ac)2Ga1(Ac)1
	A2		A2G2S(3)1		FA3G2S(3)2
GP 5	A1G1		A2B[3]G1S(6)1		FA3G3S(3)1
	FA1GalNAc[SO4-2]1		A2G1G[SO4-2]1S(3)1		A2BG2S(6)2
	FM4		FA3G2		FA2BG2S(3,6)2
GP 6	M4A1	GP 22	FA2GalNAc2S(6)1		A3G3S(3)2
GP 7	A2B		FA2BG1GalNAc[SO4-2]1S(3)1		FA2G1G[SO4-2]1S(6)2
GP 8	FA2		FA2F1G2		FA4F1G3S(3)1
GP 9	A2GalNAc2[SO4-2]2		FA2G1GalNAc[SO4-2]1S(6)1	GP 33	FA2G2S(6)2
	M5	GP 23	FA2G1G[SO4-2]1S(3)1	GP 34	FA3G3S[6]1
	FA2B		M5A2BG1	GP 35	FA2BG2S(6)2
	A2G1		FA2G2S[3]1	GP 36	FA3G3S(3)2
GP 10	FA1G1		FA2BG1GalNAc[SO4-2]1S(6)1	GP 37	A3G3S(6)2
GP 11	A2B[6]G1		FA2G1GalNAc[SO4-2]1S1		FA1A[SO4-2]1G2S(6)2
	M4A1G1		A2G2S(6)1	GP 38	FA3BF2G3S(3)1
GP 12	FA2G1		FM4A1G1Sg1S(6)1		FA3G3S(6)2
	A2B[3]G1		A3BG3	GP 39	A3G3S(6)3
GP 13	A1GalNAc1S(6)1	GP 24	A2G1GalNAc1S(3,6)2	GP 40	FA3BG3S(3)2
	FA2BG1		FA1A1[SO4-2]1G2Ga1S(3)1		FA3G3S(3)3
	A2G2		A2B2S(6)1	GP 41	A3G3S(6)3
GP 14	FA2G1GalNAc[SO4-2]1	GP 25	FA4G3		FA3BG3S(6)3
	FA2GalNAc2		FA2G2S(6)1		FA3G3S(6)3
	A1G1Sg1S(3)1	26	M8	GP 42	A3G3S(6)3
GP 15	M6 D1 or D2		M5A1G1S(6)1		FA3BG3S(6)3
	A1[6]G1S(6)1	GP 27	A2G2S(6)1S(3)Ac)1	GP 43	FA4G3GalNAc1S4
	FA1[6]G1S(3)1		M5A2G2Ga2		A3G3S(3)4
GP 16	FA1GalNAc1S(3)1		FM5A2G2Ga1S[6]1		FA3F1G3S(3)3
	FA3G1	GP 28	FA4G3	GP 44	FA4BG3S(3,6,6)3
	A1[3]G1S(6)1		FA2F1G2(Ac)2Ga1(Ac)1		FA4G4S(3)3
	FM4A1G1		FA2G1G[SO4-2]1S(6)1		FA3F1G3S(6)3
GP 17	A1GlcA1G2S1		FA2BG2S(6)4	GP 45	A3G3S(6)4
	A2BG2		A3G2G[SO4-2]1S(3)1		A4G4S(3)4
GP 18	M5A1G1	GP 29	FA2G2S(3)2	GP 46	FA4G4S(6)3
	A2G1GalNAc1[SO4-2]1S(3)1		FA2G1GalNAc1S(3)2	GP 47	_
	M4A1G1S(3)1		FA2BG2S(3)2	GP 48	A4G4S(6)4
GP 19	A2[6]G1S(6)1		FA2GalNAc2S(6)2	GP 49	FA4F1G4S(3)4
	FA2G2		FA2F1G2(Ac)2Ga1(Ac)1	GP 50	FA3GalNAc1G2S(6)3
GP 20	A2G2S(3)1Ac)1	GP 30	FA3G3S(3)1		
	FA1[6]G1S(6)1		M9		
	FA2F1GalNAc2		A3G2G[SO4-2]1S(6)1		
	FA2G1S(6)1				
	FA1[3]G1S(6)1				
	A2[3]G1S(6)1				
	FA2GalNAc1S(6)1				
	M4A1G1Sg1S(6)1				
	FA2BG2				

Figure 3. (A) Representative HILIC-UPLC chromatogram of the whole *N*-glycome profile of a control (without endometriosis) urine sample and highlighted GP36 which is the only significantly altered GP endometriosis vs controls. **(B)** Glycan structures in each GP of the whole urine *N*-glycome profile.

Structure abbreviations: All *N*-glycans have two core GlcNAcs; F at the start of the abbreviation indicates a core-fucose α1,6-linked to the inner GlcNAc; Mx, number of mannose on core GlcNAcs; Ax, number of antenna (GlcNAc) on trimannosyl core; A2, biantennary with both GlcNAcs as β1,2-linked; A3, triantennary with a GlcNAc linked β1,2 to both mannose and the third GlcNAc linked β1,4 to the α1,3 linked mannose; B, bisecting GlcNAc linked β1,4 to β1,3 mannose; Gx, number of β1,4 linked galactose on antenna; Fx, number of fucose linked α1,3 to antenna GlcNAc; Sx, number of sialic acids linked to galactose.

Whole serum *N*-glycome in endometriosis



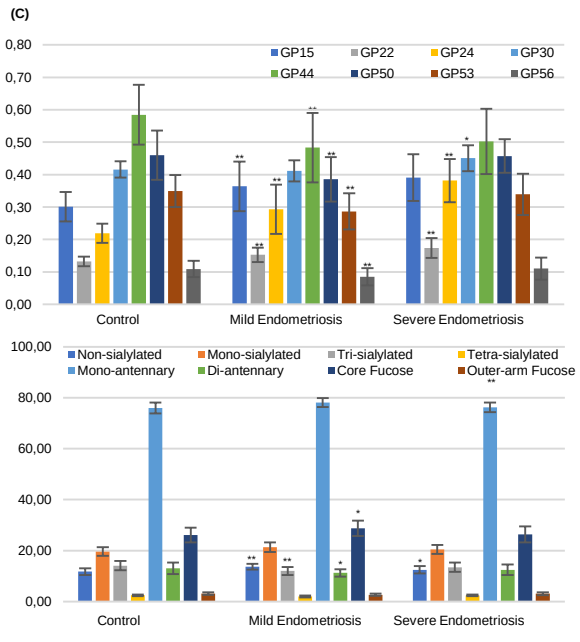


Figure 1. (A) Representative HILIC-UPLC chromatogram of *N*-glycans from all serum glycoproteins from a control (without endometriosis) sample. Glycan peaks (GP) which are significantly changed during endometriosis are highlighted with main glycans pictured. **(B)** Bar charts of the GPs, significantly altered in endometriosis in both cohorts. **(C)** Bar charts of the significantly altered GPs only in the IND cohort. Feature analysis and assignments of *N*-glycans in serum samples was done according to Saldova et al. (2). The significant alterations between the mild and severe endometriosis vs control group were plotted and marked with an asterisk. *= p<0.05; **= p<0.01).

Total *N*-glycome profile of serum and urine samples was separated using HILIC-UPLC from MFC cohort (24-control and 27-or mild and severe stages of endometriois), while total *N*-glycome of serum were analysed in case of IND study group (25-contol, 22-mild endometriosis and 23- severe endometriosis).
Urine *N*-glycome was analysed using HILIC-UPLC, exoglycosidase digestions and mass spectrometry.
Statistical analyses were performed in IBM® SPSS® Statistics software, in which control was compared with mild and severe disease groups. The glycan data were logit transformed and multivariate analysis of variance (MANOVA) with Tukey test was performed.
The MFC and IND groups overlap for many GPs, although the IND group shows significantly better results. This may be explained by the fact that the IND group contains only mid-luteal samples meanwhile the MFC cohort includes both luteal and proliferative samples, as hormones do affect glycosylation. Therefore, sampling consistency is crutial and the data indicate that sampling in mid-luteal phase is the preferable time-point.
IgG *N*-glycosylation was analyzed from all serum and urine samples. New method was developed for the whole urine and urinay IgG glycan analysis as as there was not existed protocol, which can overcome on the limitations of urine, to allow effective, reproducible analysis.

Conclusions

- The whole serum profile is the most appropriate potential source of non-invasive biomarkers.
- Serum IgG also shows differences and potential biomarker, and as an immunological glycoprotein, it could be a good target for potential diagnosis and treatment.
- A novel method was developed for extraction of *N*-glycans from non-invasively collected urine samples
- Total urine glycoproteins were analysed for detailed *N*-glycan composition for the first time.

References

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